Fluorescent *in situ* Hybridization
FISH

What is FISH

- Fluorescent *in situ* (rRNA) hybridization
- Relies on conserved and variable regions of 16S rRNA
- Uses ssDNA probes (typically 15-18 mers)
- Probes are tagged with fluorophores (e.g. FITC, Cy3 etc)
- Is done on fixed (dead) cells

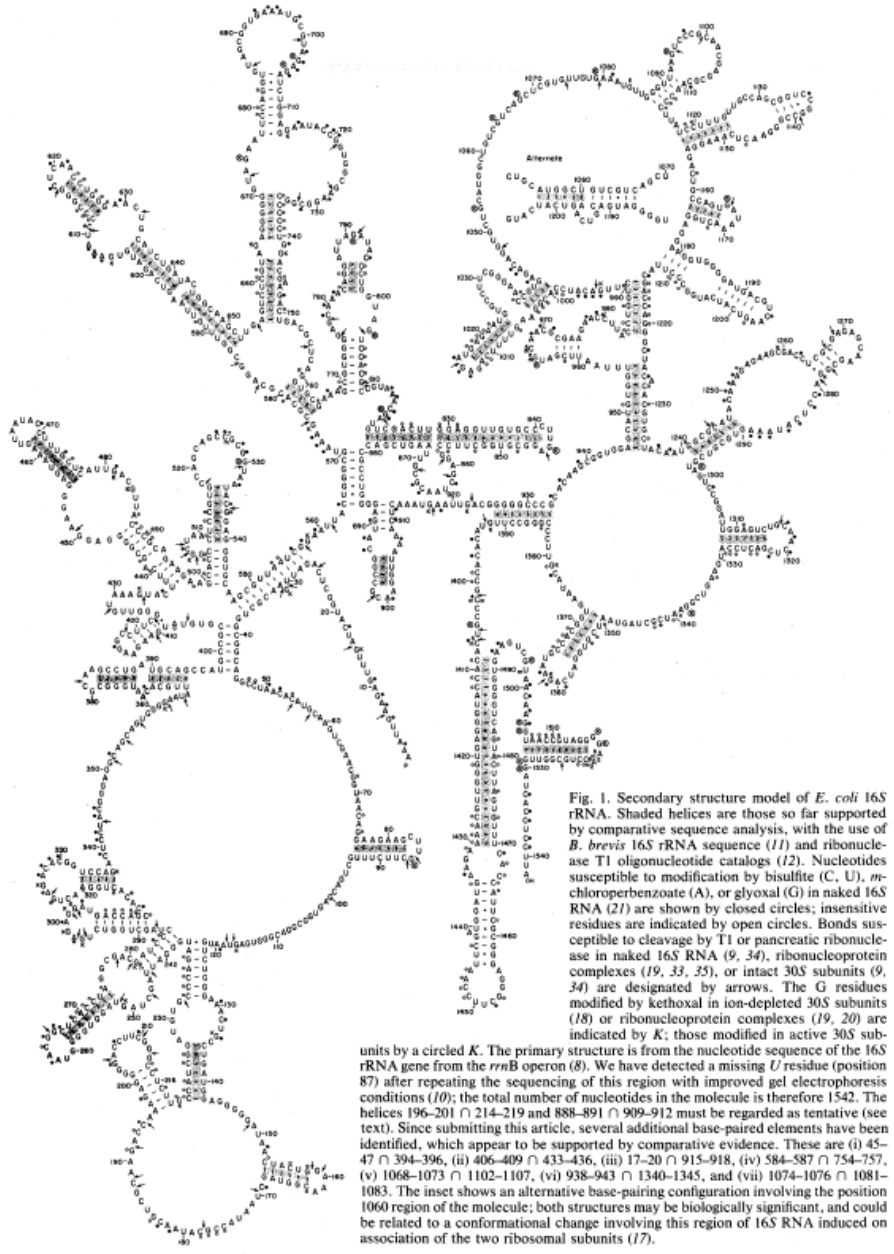


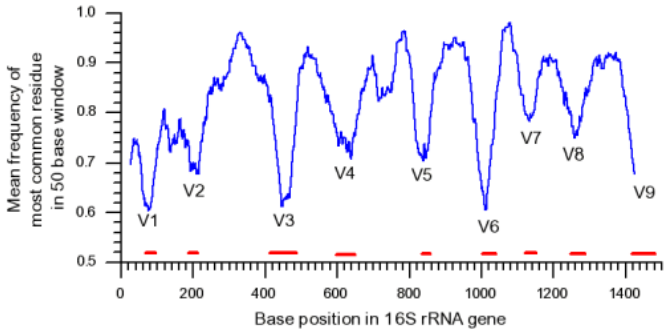
Fig. 1. Secondary structure model of *E. coli* 16*S* rRNA. Shaded helices are those so far supported by comparative sequence analysis, with the use of *B. brevis* 16*S* rRNA sequence (*11*) and ribonuclease T1 oligonucleotide catalogs (*12*). Nucleotides susceptible to modification by bisulfite (C, U), *m*-chloroperbenzoate (A), or glyoxal (G) in naked 16*S* RNA (*21*) are shown by closed circles; insensitive residues are indicated by open circles. Bonds susceptible to cleavage by T1 or pancreatic ribonuclease in naked 16*S* RNA (*9, 34*), ribonucleoprotein complexes (*19, 33, 35*), or intact 30*S* subunits (*9, 34*) are designated by arrows. The G residues modified by kethoxal in ion-depleted 30*S* subunits (*18*) or ribonucleoprotein complexes (*19, 20*) are indicated by *K*; those modified in active 30*S* subunits by a circled *K*. The primary structure is from the nucleotide sequence of the 16*S* rRNA gene from the *rrn*B operon (*8*). We have detected a missing *U* residue (position 87) after repeating the sequencing of this region with improved gel electrophoresis conditions (*10*); the total number of nucleotides in the molecule is therefore 1542. The helices 196–201 ∩ 214–219 and 888–891 ∩ 909–912 must be regarded as tentative (see text). Since submitting this article, several additional base-paired elements have been identified, which appear to be supported by comparative evidence. These are (i) 45–47 ∩ 394–396, (ii) 406–409 ∩ 433–436, (iii) 17–20 ∩ 915–918, (iv) 584–587 ∩ 754–757, (v) 1068–1073 ∩ 1102–1107, (vi) 938–943 ∩ 1340–1345, and (vii) 1074–1076 ∩ 1081–1083. The inset shows an alternative base-pairing configuration involving the position 1060 region of the molecule; both structures may be biologically significant, and could be related to a conformational change involving this region of 16*S* RNA induced on association of the two ribosomal subunits (*17*).

FISH is based on the facts that:

- All living organisms contain ribosomes made of rRNA (and proteins)
- The rRNA subunits of ribosomes (5S, 16S and 23S for bacteria) are well conserved
- Some regions of 16S are more variable than others between species and groups
- The number of ribosomes in a given cell is proportional to the maximal growth rate of that cell

Comparison of large numbers of rRNA molecules (in particular 16S) has identifed conserved and hypervariable regions.

It is possible to design a probe that is specific for a single species or a whole group or all bacteria (almost)



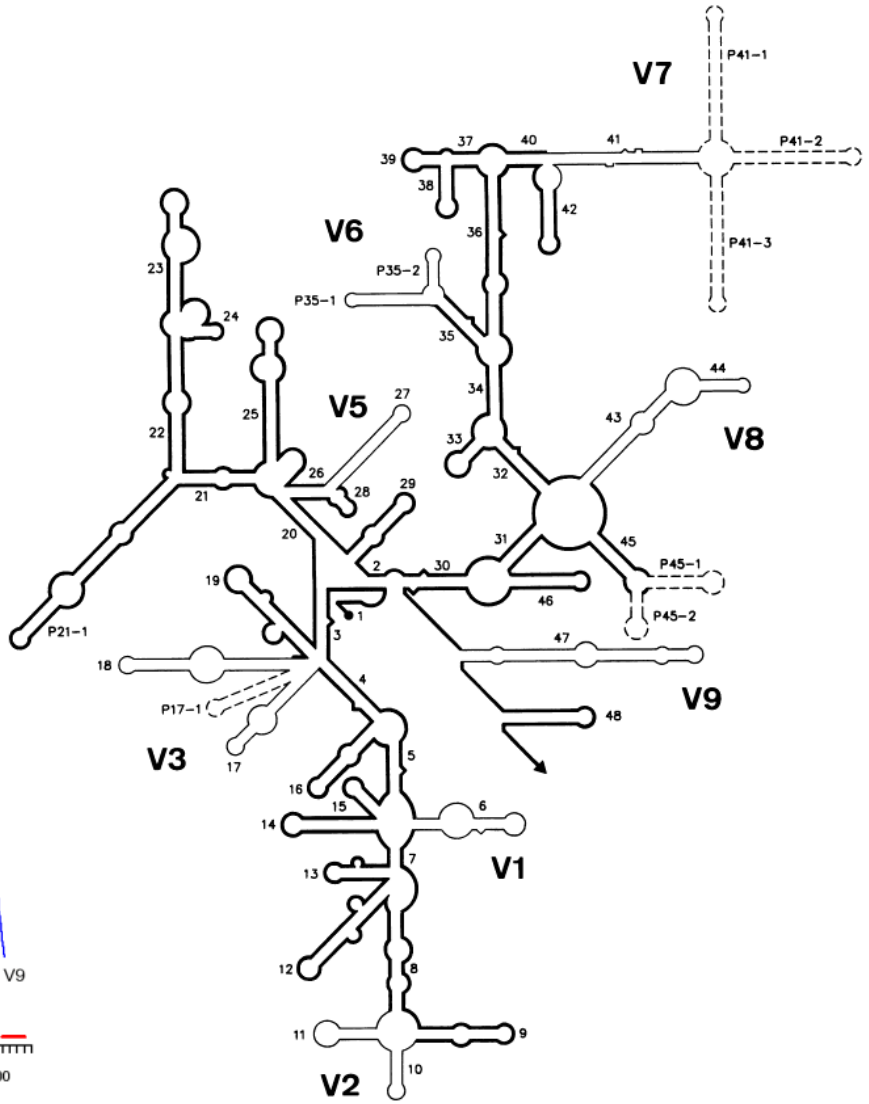


Fig. 1. Secondary structure model for prokaryotic srRNAs.
The 5′-terminus is symbolized by a filled circle and the 3′-terminus by an arrowhead. Helices are numbered in the order of occurrence from 5′- to 3′-terminus. Helices bearing a single number are common to the prokaryotic and eukaryotic (Fig. 2) models. A composite number preceded by P points to a prokaryote-specific helix. Relatively conserved areas are drawn in bold lines, areas of sequence–and length variability in thin lines. Eight variable areas, numbered V1 to V9, are distinguished, V4 being absent in prokaryotic srRNAs. Helices drawn in broken lines are present in a small number of known structures only. Archaebacterial sequences follow the prokaryotic pattern except for helix 35, which is unbranched as in eukaryotes.

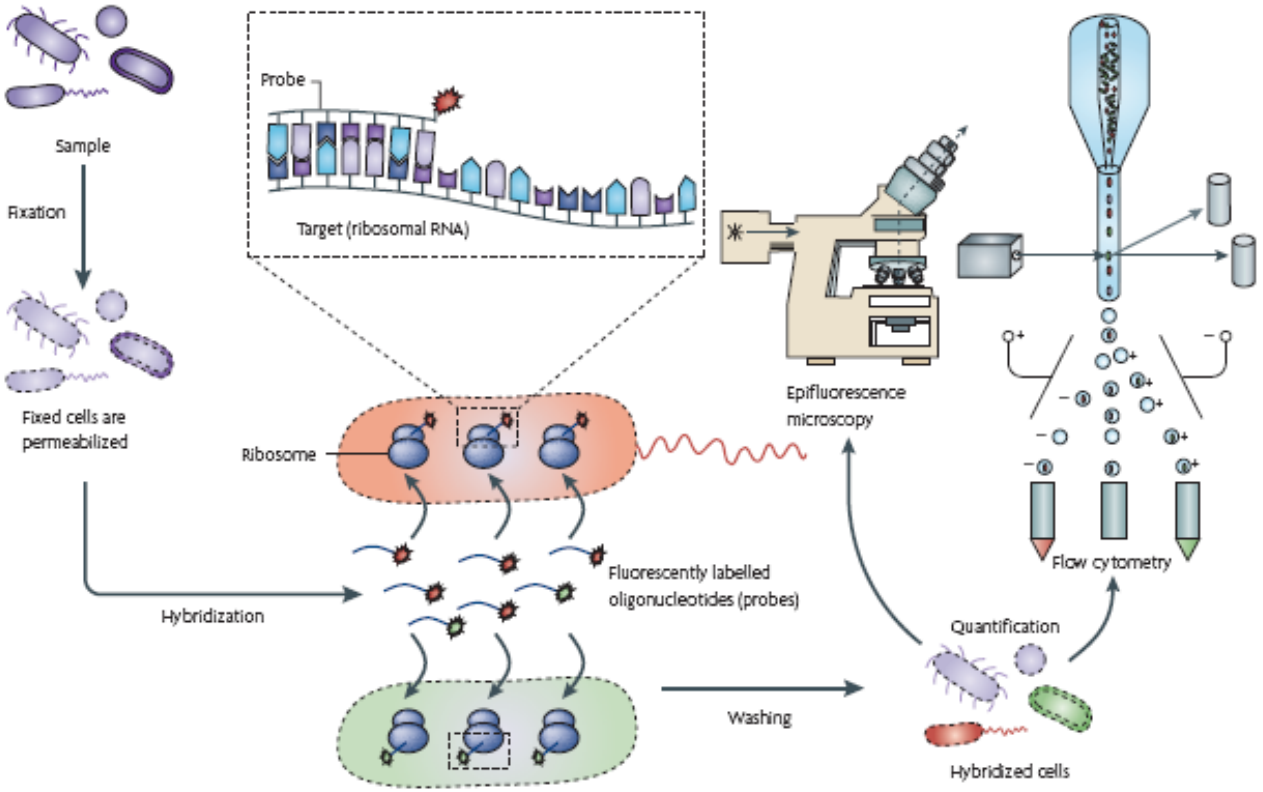


Figure 1 | Basic steps of fluorescence *in situ* hybridization. The sample is first fixed to stabilize the cells and permeabilize the cell membranes. The labelled oligonucleotide probe is then added and allowed to hybridize to its intracellular targets before the excess probe is washed away. The sample is then ready for single-cell identification and quantification by either epifluorescence microscopy or flow cytometry.

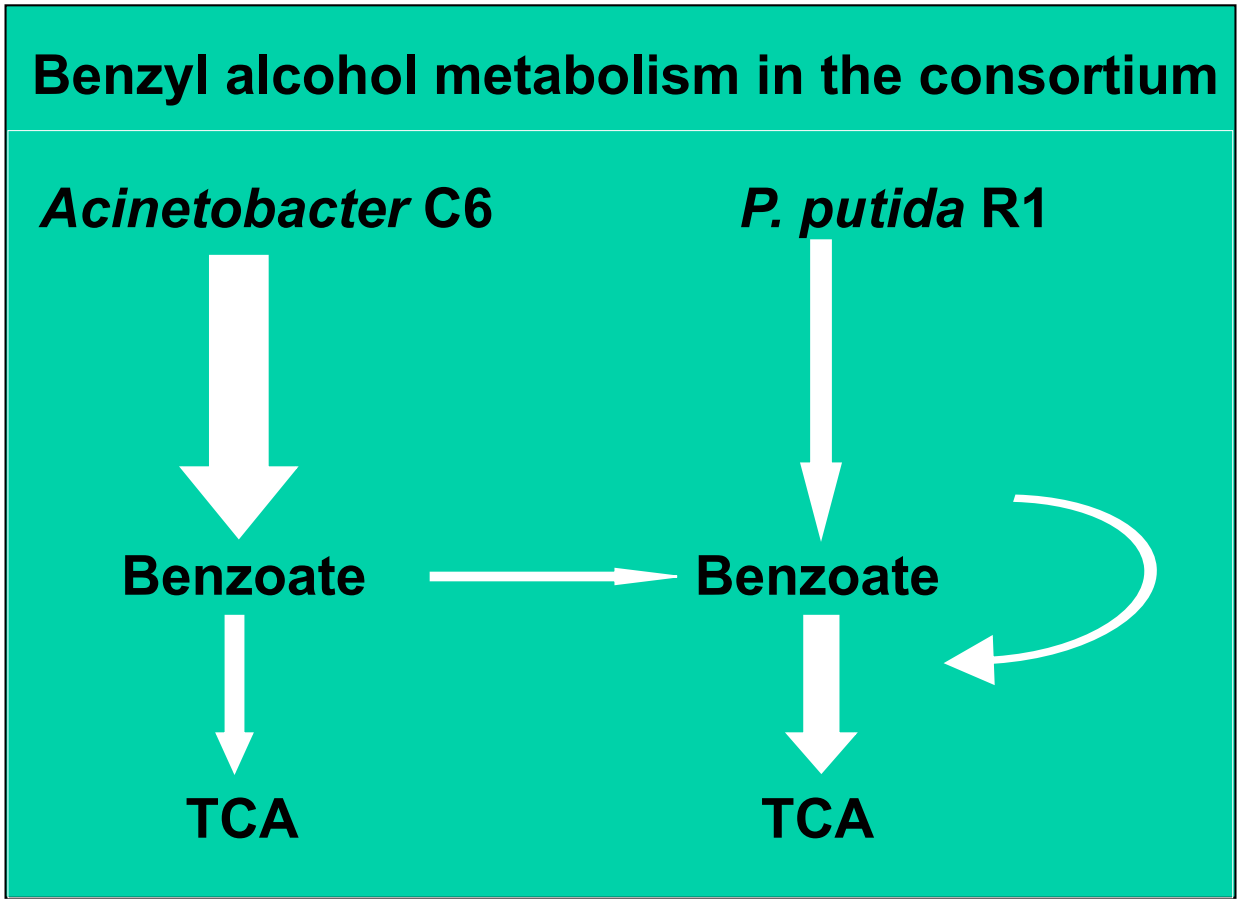
Microbial consortia investigated in flow-chambers

Acinetobacter **is leaking benzoate in mono-species biofilm.**

P. putida **R1 degrades the excreted benzoate in the two species consortium, optimizing growth of both strains.**

Two members of a natural toluene degrading community isolated from a creosote polluted site

Commensal interactions in a two species consortium



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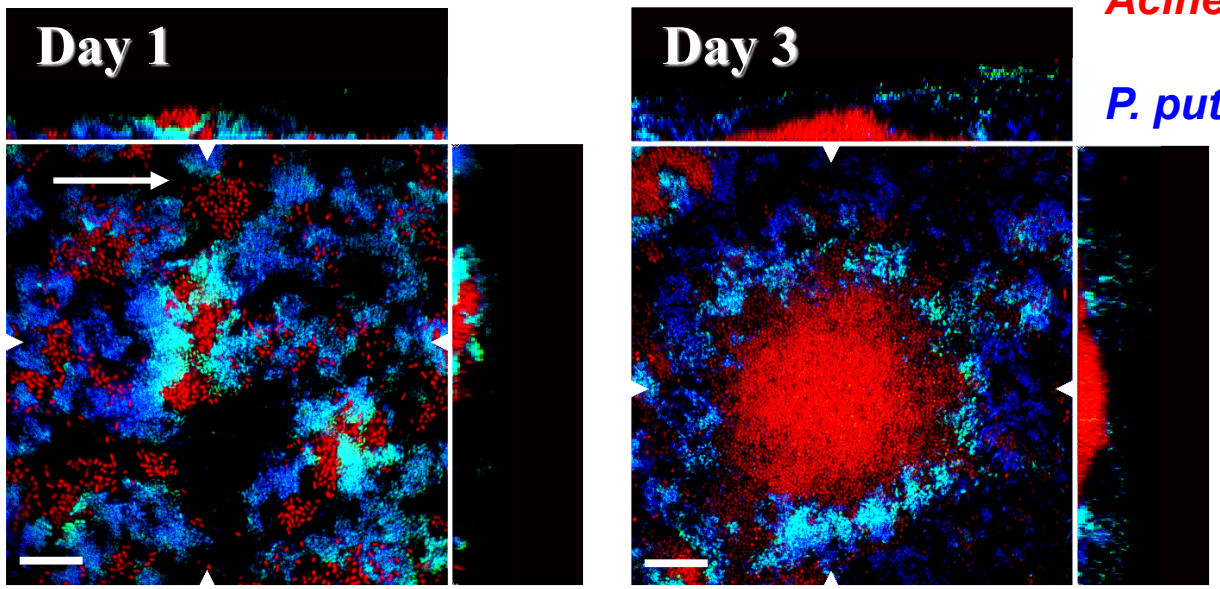


FISH (Fluorescence In-Situ Hybridization) used as phylogenetic stain (red, blue)

Gfp used as activity reporter (turquoise/green)

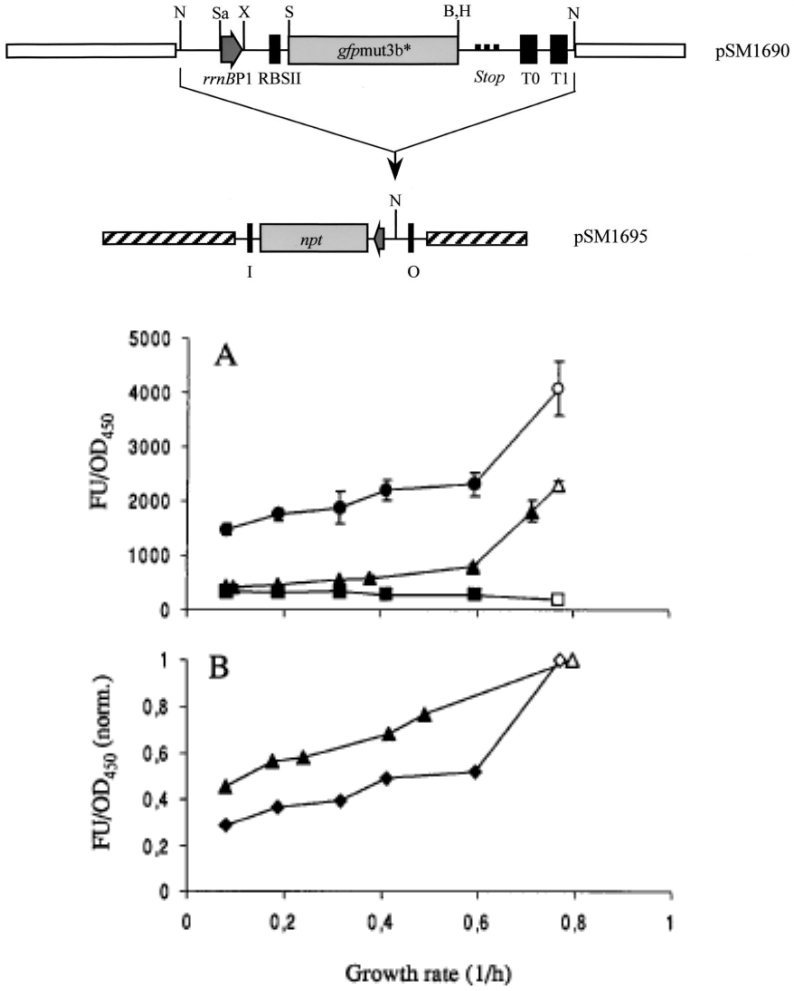
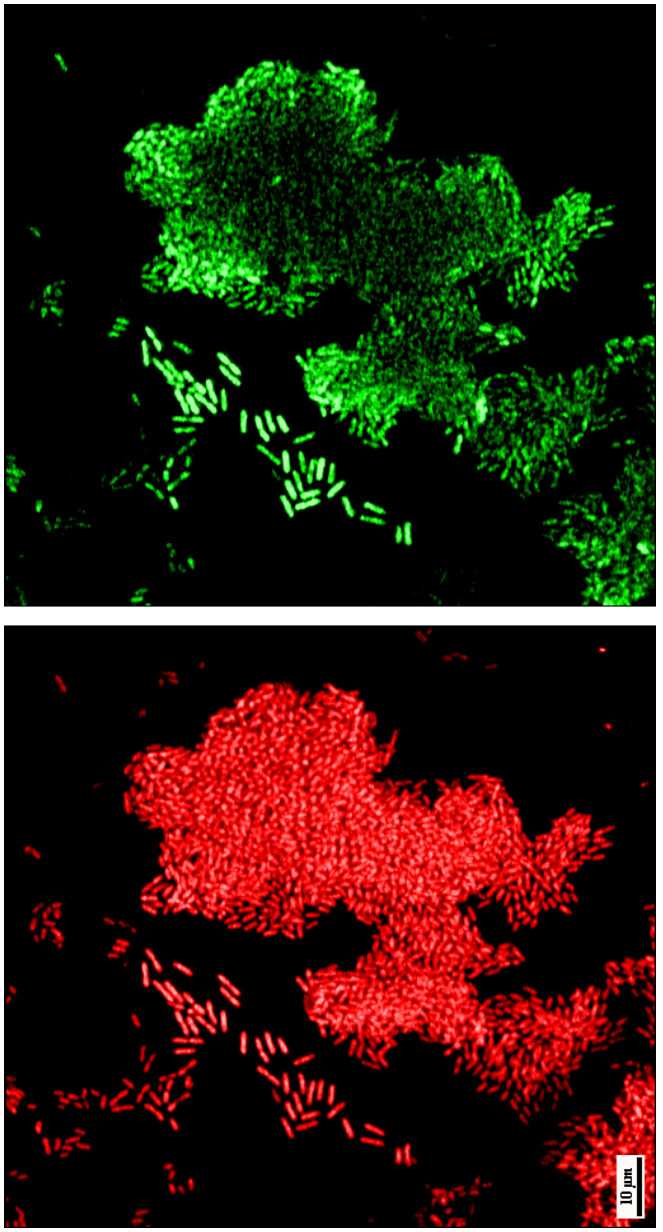


FIG. 2. (A) Variation in fluorescence intensity [wt] as a function of growth rate of strains JB156 (wild-type [wt] *P. putida* R1 [Nal^r]; ■), SM1699 (*P. putida* R1::P*rrn*BP1-*gfp*mut3b* wt; ●), and SM1639 [*P. putida* R1::P*rrn*BP1-*gfp*(AAV); ▲]. The growth rate was varied by growing cells in chemostats at different dilution rates (closed symbols) and exponentially in batch cultures (open symbols). Each point is the average of at least three independent measurements. Error bars indicate standard deviations. (B) Fluorescence intensity of strain SM1699 with the background from JB156 subtracted (◆) compared with the fluorescence intensities of 16S rRNA hybridizations of wt *P. putida* R1 (JB156) cells (▲). Fluorescence intensity is normalized to 1 for cells grown in batch cultures. The ordinate axes are the same in panels A and B. FU, fluorescence units.

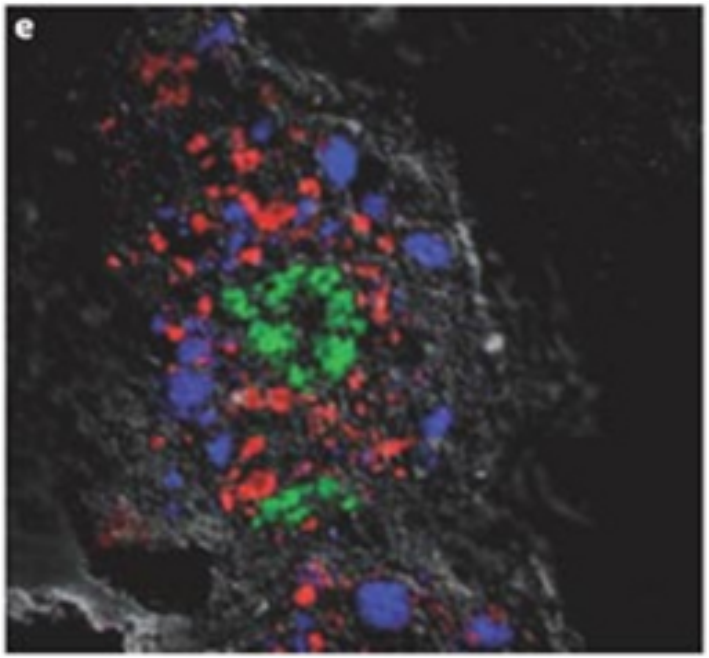


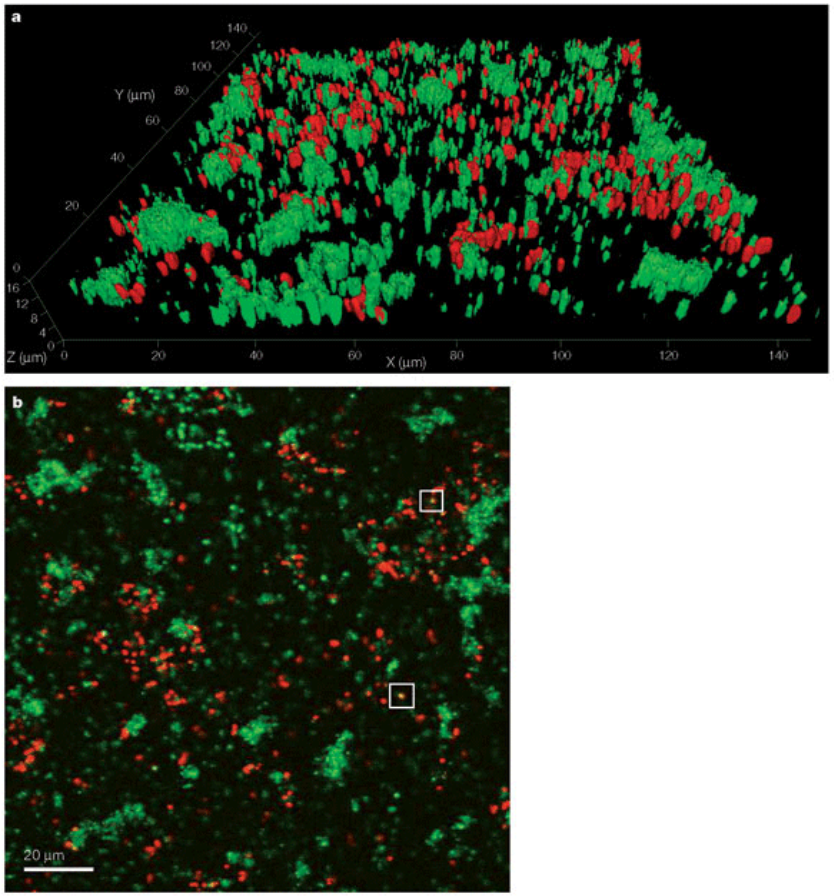
”Landscaping” bacterial species in waste water biofilms

Red: Specific probe against *Nitrospira*
Green: Specific probe agains sublineage II *Nitrospira*
Blue: Probe against Ammonia oxidizing bacteria

Structure is the consequence of nutrient gradients

Maixner et al (2006). Environ. Microbiol. 1487-1495





Using FISH and RFP to visualize horizontal gene transfer.

Green: recipient (*Delfia acidovorans*) FISH labelled with a β-proteobacteria specific probe with FITC
Red: Donor (*Pseudomonas putida*) harboring a plasmid encoding Rfp
Yellow (boxed): Transconjugants

Sørensen et al (2005) Nature Microbiol Rev 700-710

CARD –FISH

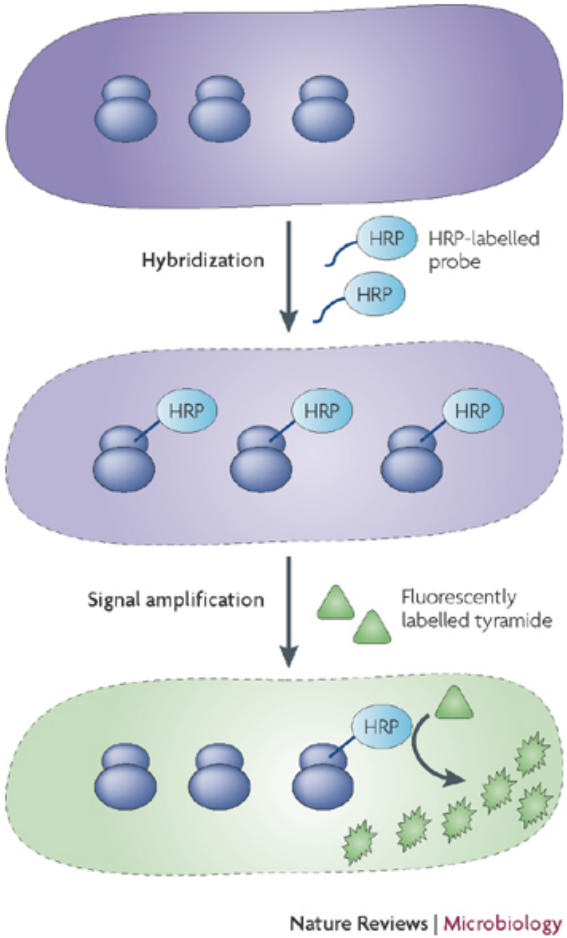
Ribosomes in fast growing cells are abundant (from 70,000 to 6,000 per cell in *E. coli* growing at generation times of 24min to 100 minutes)

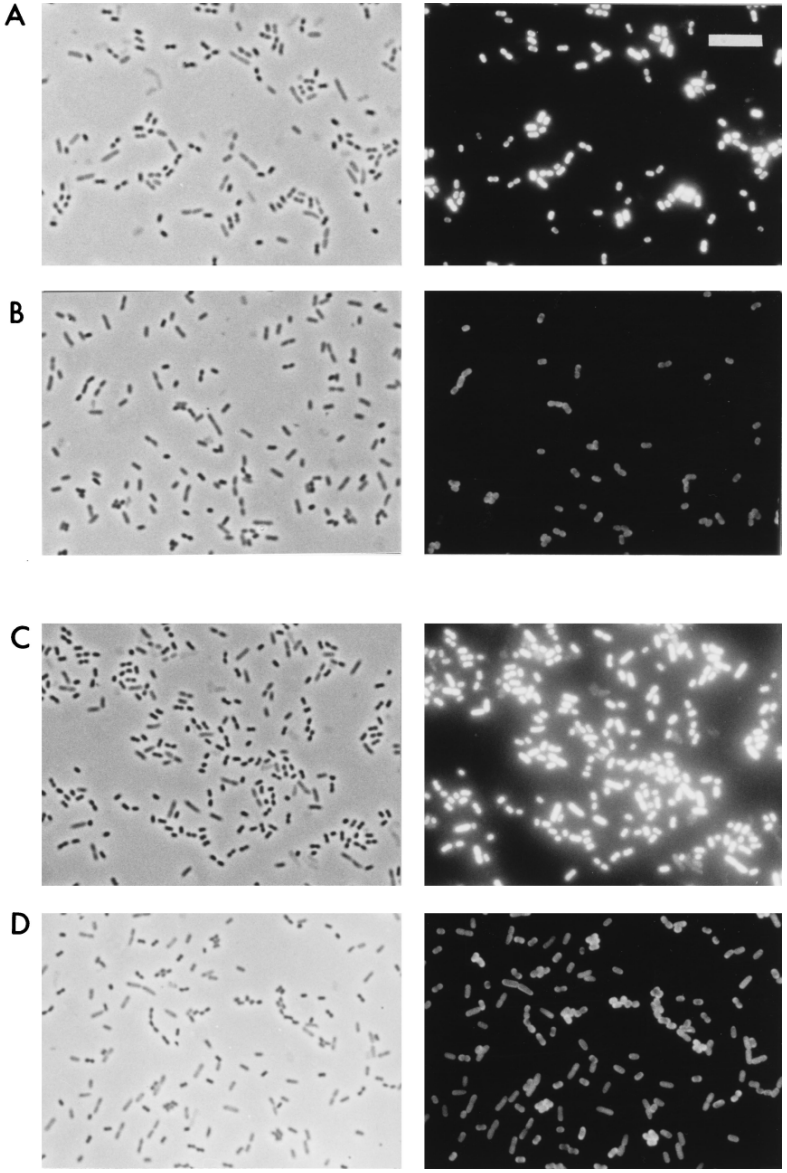
In natural isolates these numbers may be much lower, giving a very low fluorescence signal

CARD*-FISH is a new method to increase sensitivity of the FISH protocol

*CAtalyzed Reporter Depostion

Common problem: Harsh permeabilization conditions are needed, giving loss of cells in the process.





CARD-FISH example 1

Mixture of *Acinetobacter calcoaceticus* and *Escherichia coli*

A: CARD-FISH with ACA probe
B: FISH with ACA probe

C: CARD-FISH with EUB probe
D: FISH with EUB probe

Schönhuber et al (1997) Appl. Environ. Microbiol. 3268-3273

RING*-FISH

Further increase of sensitivity.

Probe is a 350-800 nucleotide RNA polynucleotide with a fluorescence label for each 20th nucleotide.

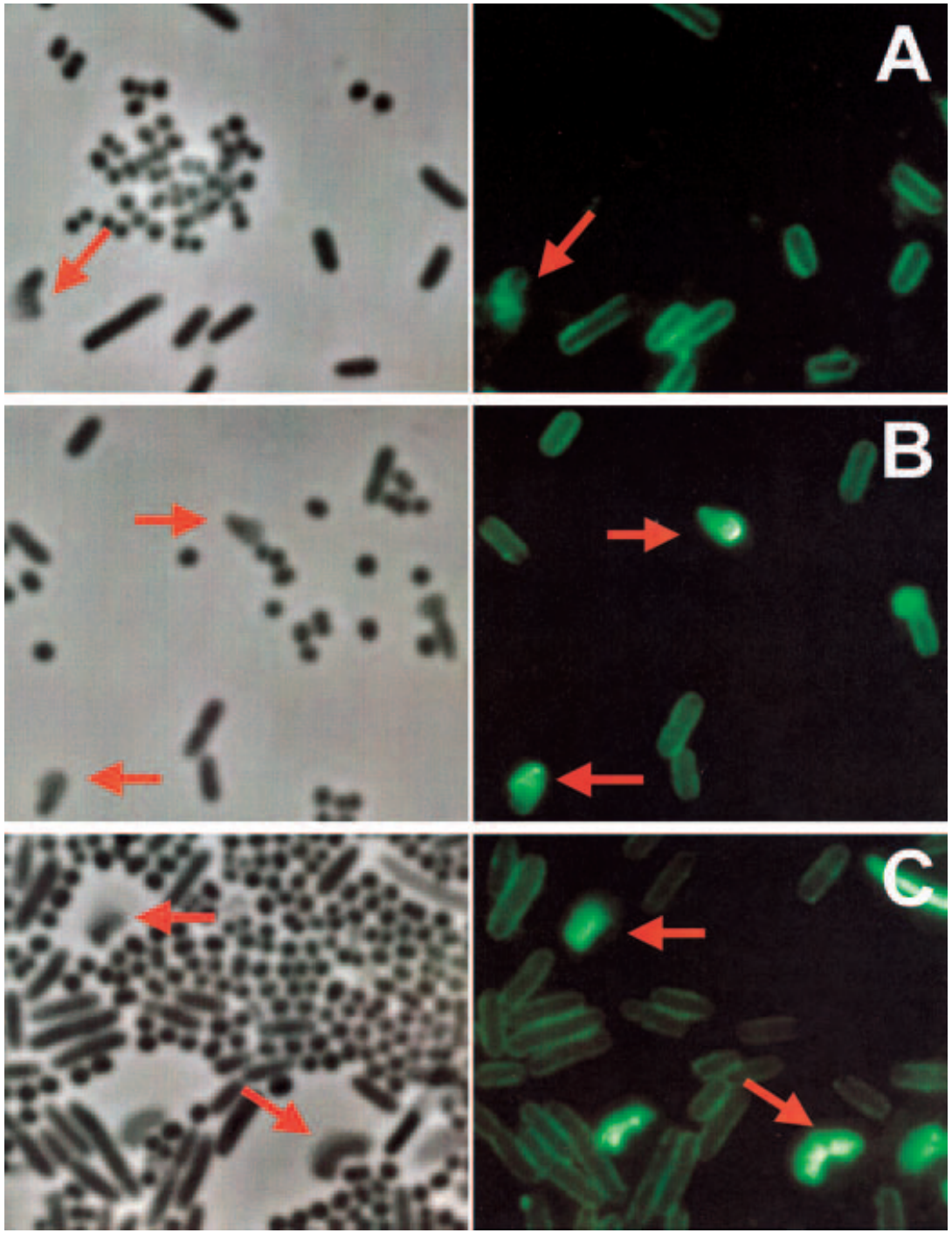
Target: betalactamase gene

A: High copy number plasmid in *E.coli (rods)* N=200-500
B: Medium copy number plasmid N=30-50
C: Low copy number plasmid N=15-20

Cocci: N. Canis

•Recognition of INdividual Genes

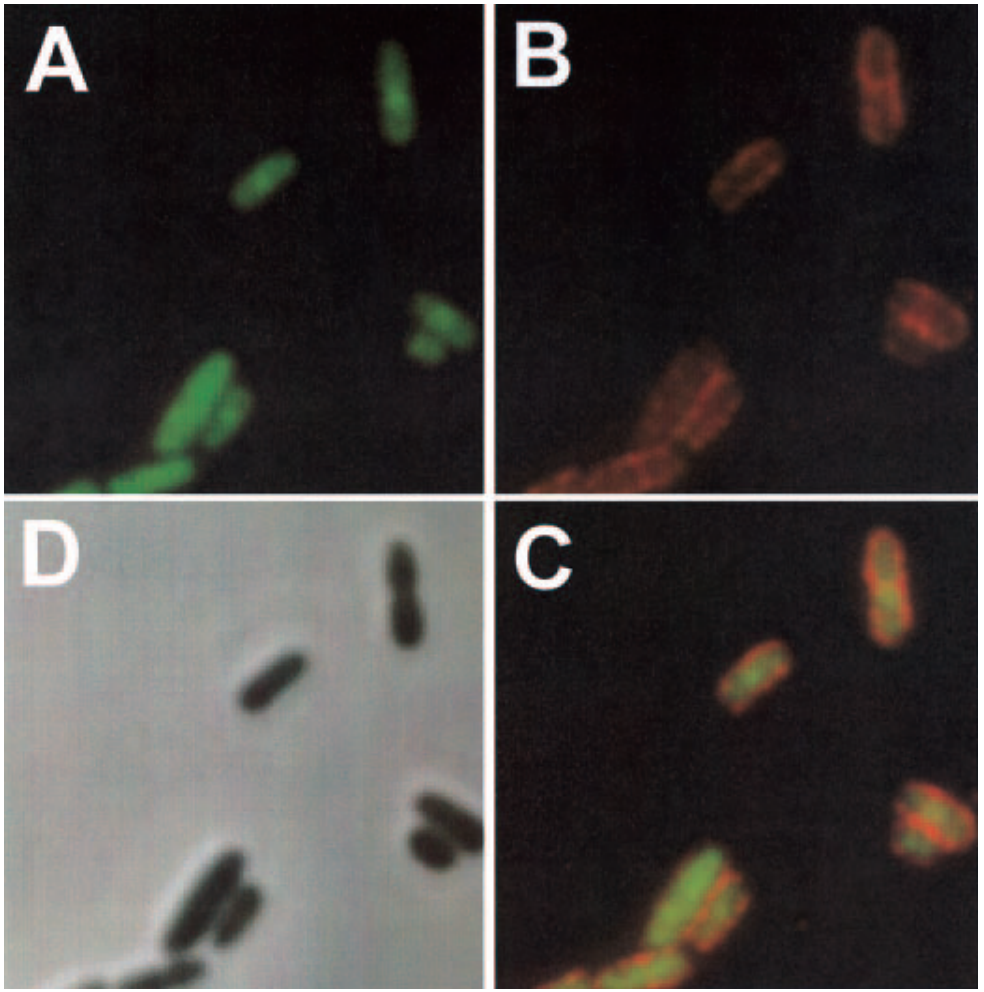
Zwirglmayer et al (2004) Molec Microbiol. 89-96



RING-FISH in combination with normal FISH

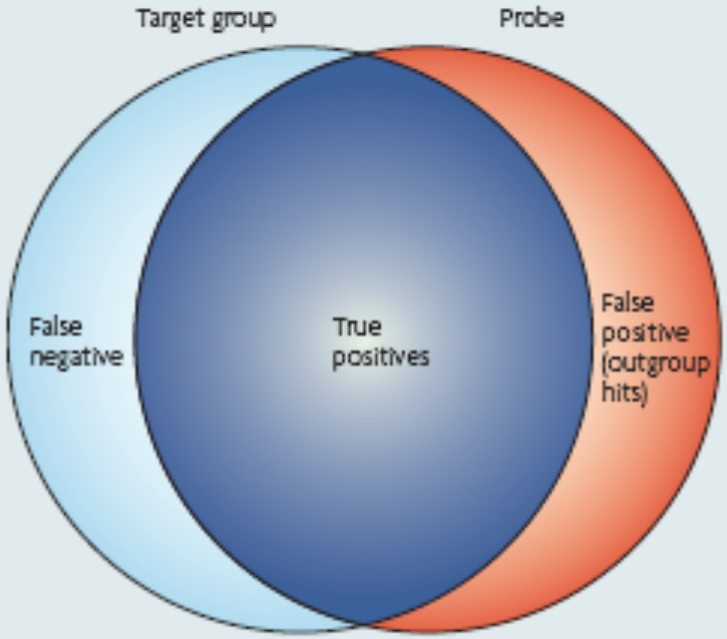
Green: EUB probe
Red: RING-FISH probe

Target for RING-FISH: low copy number plasmid
N=15-20



More knowledge means less specificity (?!)

Box 2 | **Intended target group versus specificity — nomenclature**



There is no such thing as a perfect oligonucleotide probe that only targets the target group (see the figure). In this Review, we refer to group coverage as the percentage of group members that are identified relative to the total number of members in the group. Target organisms that remain unstained are classed as false negatives. Probes also produce false-positive identifications by hybridizing to organisms that are outside the target group, and we refer to these as outgroup hits.

Target group	Probe name‡	Probe sequence (5′ to 3′)	Size of target group§‖	Number of probe hits in target group¶	Number of false negatives	Group coverage (%)#	Outgroup hits	Refs
Archaea	ARCH915	GTGCTCCCCCGCCAATTCCT	7,932	7,141	791	90	0	99
Bacteria	EUB338, EUB338-II and EUB338-III	GCTGCCTCCCGTAGGAGT, GCAGCCACCCGTAGGTGT and GCTGCCACCCGTAGGTGT	42,469**	39,726	2,543	94	0	100,44
Eukarya	EUK516	ACCAGACTTGCCCTCC	30,962	27,771	3,191	90	711	100
Betaproteobacteria	BET42a‡‡§§	GCCTTCCCACTTCGTTT	224	193	31	86	62	45
Gammaproteobacteria	GAM42a‡‡§§	GCCTTCCCACATCGTTT	993	759	234	76	4	45
Actinobacteria	HGC69a§§	TATAGTTACCACCGCCGT	152	141	11	93	1	18
Alphaproteobacteria	ALF968	GGTAAGGTTCTGCGCGTT	12,165	9,794	2,371	81	1,771	50
Bacteroidetes	CFB560	WCCCTTTAAACCCART	21,002	19,601	1,401	93	87	52
Bacteroidetes	CF319a	TGGTCCGTGTCTCAGTAC	21,002	8,050	12,952	38	1,086	51
Planctomycetales	PLA46	GACTTGCATGCCTAATCC	1,271	559	712	44	265	53
Planctomycetales	PLA886	GCCTTGCGACCATACTCCC	1,271	872	399	69	17,154	53

*A comprehensive database of >600 oligonucleotide probes that have been tested for FISH (probeBase; see Further information) is available online[55]. ‡The probe name might differ from the name that was used in the original publication. The most commonly cited name is used instead, such that the abbreviation resembles the name from both the target group and the target region according to the *Escherichia coli* numbering system of Brosius and colleagues[101]. §Number of sequences from the target group in the SILVA database (see Further information). ‖The target group is based on an ARB (see Further information) parsimony tree that includes all of the sequences from the respective database. ¶The probe match was carried out using the PROBE MATCH module of the ARB programme (for details, see Supplementary information S1 and S2 (figures)). The results are based on the comprehensive rRNA database SILVA (release 91), which contains 196,890 aligned small subunit rRNA sequences and 6,902 aligned large subunit rRNA sequences. #The number of probe hits in each target group was divided by the total number of sequences in that target group. **Based on a small subunit rRNA database from 2004 that is available from the ARB project. ‡‡Competitor probes are required. §§The target is 23S rRNA. FISH, fluorescence *in situ* hybridization; rRNA, ribosomal RNA.